Dietetic supplementation with DHA Triglyceride* and antioxidants in Evaporative Dry Eye. Our Clinical Experience

*Tridocosahexaenoine-AOX*

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Evaporative dry eye is the main cause of eye dryness. Multiple aspects can drive to a lipid production of a low quality in tears. Both menopausal hormonal changes and posterior blepharitis are responsible for the production of a Meibomian sebum of a low quality. In Posterior blepharitis there exist a Meibomian gland disfunction; the lipid produced is too dense, it turns easy to get glued inside the gland or the duct as a Chalazion, favoring an accelerated evaporation of the aqueous phase in the tear film. Dry Eye is more prevalent in women over 50 years of age. Treatment is based on the use of artificial tears, trying to improve hydration and lubrication of the ocular surface. Today we know that the type of diet we perform is strongly influencing the ocular surface physiology. By reducing Omega-6 Poly-Unsaturated Fatty Acids (ω6-PUFA) consumption, showing a pro-inflammatory profile, and increasing ω-3 PUFA consumption, offering an anti-inflammatory profile, plus increasing also consumption of some antioxidants as vitamins A, C and E, we can contribute improving the ocular surface health. Different clinical trials performed by BRUDYLAB are demonstrating how important is supplementation to mitigate both the inflammation and the bothering dry eye symptoms.

PHYSIOPATHOLOGY AND PREVALENCE OF EVAPORATIVE DRY EYE

Lipids in general are hydrophobic. Can’t mix with water, and are less dense, floating on it, creating a non-homogeneous dispersion. For this reason in the tear film exists the need to establish a polar lipid phase, capable to reduce surface tension and to establish hydrogen bonds with the aqueous phase of the tear film located just beneath. By this means is created a stable lipid phase, capable to extend itself covering completely the surface of the aqueous phase in a homogeneous manner.

The polar lipid phase is constituted by a monolayer of lipids with electric charge, corresponding to the phospholipids of the cell membranes from the Meibomian acini. This thin polar phase is responsible for holding the much thicker non-polar lipid phase, which is situated over, exerting an anti-evaporative effect. Its basic function is to stabilize the thicker non-polar phase. Non-polar lipids correspond to the sebum constituents of the lipids contained in the cytoplasm of the Meibomian acini. When the polar lipid-phase is of a bad quality, the tear film lipid breaks in an accelerated form (Break Up Time: B.U.T.) favoring quick evaporation and desiccation of the ocular surface.

Non-controlled with artificial tears, ocular surface dryness (hydrating and lubricating tear substitutes) is responsible for a progressive desepithelialization and squamous metaplasia (pathologic transformation) of the mucus epithelium in the conjunctiva. Mucus producing chaliciform cells disappear, and lactoferrin, a protein acting as a health marker of the ocular surface, is reduced.

LIPIDS OF THE TEAR FILM

Meibomian glands are specialized sebaceous glands, which are organized in parallel situation both in the upper and lower eyelids. Drainage orifices from their ducts are located just behind the eyelashes, in the posterior margin of the eyelid. Constant blinking does “milk” the glands to get the sebum fall into the ocular surface, extending itself on top of the aqueous phase of the tear. Meibomian disfunction results in a sebum production of bad quality, which permits an accelerated, tear aqueous phase evaporation and thus, concentration of its mineral salts, resulting in an hypertonic tear (increased osmolality). Constant exposition of the cornea and the conjunctiva epithelium to osmolality in excess it harms the cells of the ocular surface and activates the irritating-inflammatory process.

MEIBOMIAN GLANDS AND MEIBOMIAN DISFUNCTION

Dietetic supplementation with DHA-Triglyceride® plus antioxidants in evaporative Dry Eye

Dry Eye Syndrome is affecting 3.2 million of american women over 50 years¹

Dietetic supplementation with DHA Triglyceride and antioxidants in Evaporative Dry Eye.
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04. MEIBOMIAN HOLOCRINE SECRETION

We can see a frozen section of a Meibomian gland. Built of multiple accini, just as a grape cluster, voiding their sebum content into a central duct. Each duct drains in the posterior lid margin. It is a holocrine external secretion gland. In the microphotograph we can see some accini full of cells. Young germinal cells are located at the periphery of the accini, and the cell membrane (made of polar phospholipids) breaks liberating the non-polar sebum content of the cytoplasm. Both type of lipids fall on to the tear of the ocular surface.

05. EVAPORATIVE DRY EYE: TEAR HYPEROSMOLARITY

As mentioned, accelerated evaporation of the aqueous phase of the tear film is favored by the bad quality of both lipid layers. It induces an hypertonic tear and an epithelial harm, shown as inflammation in the ocular surface. A post-menopausal androgenic deficit can be associated to posterior blepharitis or to an excessive exposition of the ocular surface to the environment due to a decreased blinking frequency. This last aspect can be seen in people working an excessive time reading or looking at video-screen terminals which induces a lower blinking frequency, reducing hydration and lubrication of the ocular surface. In all this cases it appears bothering symptoms associated to ocular dryness: stinging, burning, foreign body sensation, heavy lids sensation, pain, redness, blurred vision,…

06. ROLE OF OMEGA-3 ESSENTIAL FATTY ACIDS IN DRY EYE

Omega-3 PUFA as docosahexaenoic acid (DHA) are obtained from our diet and transformed into phospholipids, being deposited in all the cell membranes of the body with the mission to confer them fluidity and flexibility, based on their richness in double bonds. DHA is the most polyunsaturated fatty acid; it carries 6 double bonds, and is the one that confers a higher degree of fluidity to cell membranes. Diet rich in blue fish offers a large quantity of Omega-3 PUFA as DHA. It’s an essential fatty acid for humans, because we can’t produce them by modifying glucose molecules. We don’t have the necessary enzyme to introduce the first double bond in the third position of the carbon chain, from the Omega carbon. For this reason we have to obtain them from the diet, either in their final chemical form, or as a precursor. As a result of cooperating in research between BRUDY and the Biochemistry and Molecular Biology Department at University of Barcelona, we were able to get a patent on the powerful cell antioxidant effect offered by pure DHA triglyceride (DHA-TG), known biochemically as Tridocosahexaenoic. By offering this chemical form, cells get highly enriched in DHA, suffering an up-regulation on the enzymes responsible for cytoplasm glutathione synthesis. Glutathione is the main antioxidant molecule produced by vertebrate (and mammal) cells enabling us to survive in the rich in oxygen earth atmosphere.
All the fatty acids are built from a larger or shorter backbone chain of carbons, more or less saturated with hydrogen. Double bonds confer them a more fluid structure. We find them in the blood organized as lipoproteins, triglycerides or phospholipids. Glycerides are built from a glycerol molecule (an alcohol) attached to one, two or three fatty acids, constituting mono, di or triglycerides. When a polyunsaturated is located in the central Sn-2 position of the triglyceride, it is normally transformed into a phospholipid and inserted into a cell membrane. To do so, the fatty acid located in the Sn-3 position is liberated and substituted by a nitrogen base and a phosphate group, which have polarity. With such structure, phospholipids inserted into cell membranes organize themselves as a phospholipid bilayer; this is how we find them in all the organ cells.

Besides the capacity of ω-6 and ω-3 PUFA to confer fluidity and flexibility to cell membranes, other properties as pro-inflammatory or anti-inflammatory activity should be highlighted depending on their predominance. If ω-6 PUFA, as arachidonic acid (ARA), predominates, a pro-inflammatory activity will develop. But if ω-3 PUFA predominates as EPA or DHA, activity will predominate as anti-inflammatory. Today our diet is much more rich in ω-6 PUFA rather than in ω-3 type. So we can get profit of it to influence the type of phospholipids we want to be deposited into our cell membranes. If we increase the consumption of ω-3 PUFA, but at the same time we reduce ω-6 PUFA consumption we can mitigate ocular surface inflammation in patients with dry eye, especially in the evaporative type.
Dietetic supplementation with DHA Triglyceride and antioxidants in Evaporative Dry Eye.

Our Clinical Experience

In this graphic we can see that a long term rich in Omega-3 PUFA diet in humans is responsible of increasing their levels both in red blood cell membranes and in serum after 12 months of supplementing. At the same time we can see how ω-6/ω-3 ratio keeps reducing during a 12 months period. This is due to the increasing of the denominator (larger amount of ω-3 PUFA) and a decreasing in the numerator (less amount of ω-6 PUFA) in the ratio. It shows clearly how the type of diet permits modifying the phospholipid content in cell membranes, in favor of ω-3 PUFA, reducing the inflammatory potential.

10. A RICH IN ω-3 PUFA DIET DOES ENRICH THE CELL MEMBRANES

Fusion point of the fatty acids, and thus their fluidity depends on the amount of double bonds they incorporate. DHA is the most unsaturated and fluid fatty acid known (6 double bonds), with a fusion point of -20º C. In this graphic we can see how the fusion point decreases depending on the amount of double bonds present. Saturated fatty acids without any double bond show themselves solid at a corporeal temperature (steanic, palmitic, eucic fatty acids). Due to this fact, a prolonged rich in DHA diet permits its incorporation in all the cell membranes of the body conferring them fluidity and flexibility.

11. FLUIDITY DEPENDS ON THE AMOUNT OF DOUBLE BONDS

DHA-triglycerides transformed into phospholipids are inserted into the cell membranes. Their hydrophobic area, which correspond to the fatty acids, are situated in close contact with the hydrophobic zone of the opposite layer of phospholipids (the inverted layer). Contrary, the polar heads (hydrophilic) of both phospholipid layers are located, in close contact with the extracellular fluid the external one, and in close contact with the intra-cytoplasmic fluid the internal one. With such organization of a phospholipid bilayer, cytoplasmic content of the cell is insulated from the surrounding extracellular fluid creating a self-container of organelles. DHA does modulate the cell signaling. Both the cell entering chemical messages proceeding from the surrounding cells, and the cell leaving messages should cross the cell membrane.

12. DHA AS A MEMBRANE PHOSPHOLIPID MODULATES CELL SIGNALING
BRUDY has patented a DHA-Triglyceride molecule as a potent cell antioxidant. Mixing glycerol and enzymes with a DHA concentrated fish oil, having completely eliminated all saturated fatty acids, the ω-6 PUFA, and all ω-3 PUFA which are non useful to humans, we get a pure DHA-triglyceride. The triglyceride is including DHA in each one of the 3 possible attachments of the molecule. The triglyceride chemical form optimizes its digestive absorption and bioavailability. Human enterocytes better absorb triglycerides and phospholipids. This is the chemical forms by which fatty acids are present in nature, in animal meat and grease. Presence of DHA occupying the Sn-2 position of the triglyceride facilitates its conversion into a phospholipid, and favors its deposition into the cell membranes. The response of the cells to a membrane DHA enrichment (easily oxidized due to its 6 double bonds) is an up-regulation (activation) of the 3 enzymes involved in glutathione synthesis inside the cytoplasm. Cells then produce between 200 to 300% more glutathione than normal. The cell becomes shielded in front any kind of internal or external oxidative stress.

The glutathione role is to eliminate the formation of oxygenated free radicals, as superoxide anion, hydrogen peroxide and hydroxyl radical, which are capable of oxidizing DNA, proteins and lipids from the cell organelles. It’s an antioxidant, a pure electron donor, is a small protein built from three different amino acids: Glycine, Cysteine and Glutamic acid. Cysteine carrying SH groups is essential.

13. **BRUDY’S DHA-TRIGLYCERIDE IS A POTENT CELL ANTIOXIDANT**

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14. **DHA DOUBLE ANTI-INFLAMMATORY ACTION**

1. **DHA COMPETES WITH OMEGA-6 PUFA**

It is known that when exists a cell lesion, membrane phospholipids are metabolized by A2-Phospholipase, and by Ciclooxigenases-2 or Lipooxigenases. Metabolic derivatives from ω-6 PUFA, as ARA, are Eicosanoids-Prostaglandins and Thromboxanes E2, and Leukotrienes of the 4 series, showing intense proinflammatory, prothrombotic, quimiotactic activities. Metabolic derivatives of ω-3 PUFA are prostaglandins and thromboxanes of the E3 series and leukotrienes of the 5 series, showing anti-inflammatory, antithrombotic and mild chemotactic activities. A diet rich in ω-3 PUFA (EPA, DHA) can displace ω-6 PUFA (ARA) from the cell membranes, mitigating inflammation. This explains their utility in chronic inflammatory diseases, when reduced corticosteroid and NSAID doses are desired.

15. **DHA DOUBLE ANTI-INFLAMMATORY ACTION**

2. **DHA INHIBITS ACTIVATION OF NF-kB NUCLEAR FACTOR**

Today is known that DHA, as well as NSAID and Corticoids inhibit activation of NF-kB nuclear factor. The activation of it induces translocation towards the nucleus of the cell, triggering the transcription and synthesis of additional cytokines and chemokines (6,8,10) plus intercellular and vascular adhesion molecules (ICAM, VCAM. The result is the amplification and intensification of the inflammatory response. Some other stimuli besides oxidative stress are capable of activating the nuclear factor. But as already mentioned, DHA induces glutathione synthesis inside the cells; and glutathione is the most effective endogenous cell antioxidant; this explains the inhibitory effect of DHA over the activation of the nuclear factor. These are the two ways by which DHA is blocking inflammation.
**Ocular Surface Oxidative Stress**

- Human tears are enriched in antioxidants: cysteine, tyrosine, glutathione, ascorbic acid, uric acid,...
- Ascorbic acid offers anti-inflammatory and healing properties at a cornea level.
- Tear ascorbic acid levels are reduced after Ocular Surface surgery.
- Oral ascorbic acid supplementation induces its concentration increase both in serum and tears.
- Both tears and aqueous humor ascorbic acid levels are related with its dietetic ingestion.

1. Delalme NA; Ascorbic acid and the eye; Subcell Biochem 1996; 25:313-29

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**16. Tear Antioxidant Levels Are Influenced by Our Diet**

Ocular surface is completely exposed to environmental oxidative stress, including photo-oxidation. This explains the high content of antioxidant molecules present in the tears which are bathing the cornea and conjunctiva: glutathione, ascorbic acid, uric acid, cysteine, tyrosine, between some other molecules. Ascorbic acid is also playing an important role stimulating collagen synthesis and promoting healing. It’s known that anterior segment ocular surgery is responsible for a prolonged and significant reduction on the tear levels of ascorbic acid, and that by supplementing it orally the tear levels can be effectively increased. For this reason it should be recommended ascorbic acid supplementation, at least one week before surgery in order to get higher levels in the tears, to face surgery in much better conditions.

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**17. Ocular Surface Surgery Reduces Tear Ascorbic Levels**

Ocular surgery, basically refractive surgery does promote an important decline in the ascorbic acid tear levels, which is the main ocular surface antioxidant protector. It can be seen that in the fifth postoperative day, ascorbic acid levels are still under the levels detected in the preoperative period, no matter the type of refractive surgery is applied (PRK, Transepithelial PRK, or LASIK). Ascorbic acid can’t be synthesized by humans, and for this reason it should be obtained from our diet. For this reason it should be recommended it’s administration either orally or topically before surgery in order to protect the ocular surface from surgical oxidative stress, and also favoring the healing process.

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**18. A Rich in Ascorbate Diet Does Elevate Its Levels in Serum and Tears**

We can appreciate the effectiveness of ascorbic acid oral supplementation during 30 days. It can be seen a significant increase at serum and tears level with a daily dose of 1g. There exist a ceiling concentration of ascorbic acid in the tears of around 40μM, which can be reached after 2 days of supplementation. The response is more effective when the initial levels are low.
Initial signs of supplementation with antioxidants benefits in Dry Eye patients

- Eye surgery induces a reduction in the tear ascorbic acid levels (Bilghian A, 2001)
- Some trials show an improvement in tear stability after supplementing with antioxidant vitamins in healthy volunteers, in diabetic patients, and in Dry Eye patients.

2. Reponis V, et al; Conjunctival and tear film changes after vitamin C and E administration in non-insulin dependent diabetes mellitus; Med Sci Monit 2004; 10(S):CR231-3

There is a significant improvement in Ocular surface parameters of diabetic patients after 10 days of supplementation with C and E vitamins: Improvements in Schirmer test, B.U.T., nitrates concentration, tenein test and conjunctival cytology (Goblet cells and squamous metaplasia).


There is a significant improvement of the Goblet cell density and conjunctival squamous metaplasia of vitamin C and E supplemented diabetic patients.


19. ANTIOXIDANT VITAMINS AND THE OCULAR SURFACE
First symptoms of a positive influence of a dietetic supplementation with antioxidants on Dry Eye were detected in some trials in which antioxidants as Vitamin C and vitamin E were administered to healthy volunteers, as well as in diabetic patients and also in Dry Eye patients. In general they demonstrate offering an improvement in the tear stability (in B.U.T.), but also in the cytology of the conjunctiva, on the cornea and conjunctiva staining, and on the Schirmer test.

20. SYMPTOMS AND CLINICAL SIGNS OF DRY EYE ARE IMPROVED BY SUPPLEMENTING ANTIOXIDANTS
After 10 days of supplementation with vitamins C and E in diabetic patients suffering Dry Eye, it can be appreciated an improvement in the variables related with a healthy ocular surface. There exist significant improvements both in the tear stability (B.U.T.) and in the Schirmer test.

21. ANTIOXIDANTS SUPPLEMENTATION IMPROVES THE CONJUNTIVAL CYTOLOGY
An improvement in the ocular surface conjunctival cytology is obtained with antioxidant supplementation in non-insulin dependent diabetic patients with Dry Eye. Few days after initiating supplementation, a significant improvement in squamous metaplasia of the ocular surface can be detected, together with a recovery in the Goblet cells density (cells producing mucus in the conjunctiva).
Dietetic supplementation with DHA Triglyceride and antioxidants in Evaporative Dry Eye.

Our Clinical Experience

OMEGA-3 PUFA INFLUENCES ON TEARS AND DRY EYE

In the first decade of the 21st century appeared the first publications pointing towards a protecting role of the Omega-3 PUFA in Dry Eye, either alone or associated with vitamins and minerals. It has been used fish oil, an also Flaxseed oil because of its richness in α-Linolenic acid (40% aprox). Some other oils with variable ω-3 PUFA richness have been used including mixes with ω-6 PUFA. In recent days rich in DHA concentrates have been available offering higher contents in DHA than any other ω-3 PUFA. Today, DHA concentrates in triglyceride chemical form are available; they are enzyme synthesized, and of a maximum purity.

22. OMEGA-3 PUFA INFLUENCES ON TEARS AND DRY EYE

23. A DIET RICH IN ω-3 PUFA MODIFIES THE MEIBOMIAN POLAR LIPID PROFILE

Sullivan (2002) was aware that a rich in ω-3 PUFA diet was influencing the chromatographic polar lipid profile obtained from the Meibomian secretion of women suffering Sjögren Syndrome. Chromatogram was detecting a single polar lipid profile in those patients with a higher ω-3 PUFA consumption (besides other nutrients as Piridoxine and vitamin D) against a multiple polar lipid profile which was detected in low consumers of ω-3 PUFA. This was inducing to suspect that a larger ω-3 PUFA consumption was inducing a decline in certain type of inflammatory cytokines production present in tears.

24. A DIET POOR IN ω-3 PUFA FAVORS THE RISK OF SUFFERING DRY EYE IN WOMEN

Some trials, as the one from Cermak JN (2003), was pointing to a low ω-3 PUFA consumption in general, and of DHA in particular, as a possible cause favoring Sjögren Syndrome in women, when it was compared with the one done by a healthy control group of women. There were significant differences in both circumstances.
Recent trials done with larger samples of participants as the Women’s Health Study by Miljanovic (2005) studying the risk of suffering Dry Eye by means of a self obtained food frequency questionnaire (women volunteers as nurses and doctors) was confirming that the larger is the amount of fish portions (or DHA) weekly consumed, the lower is the risk of suffering Dry Eye; in a dose dependent manner (per week portions of fish consumed).

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**THE WOMEN’S HEALTH STUDY**

Based on these results, and on some other publications, which refer having obtained positive results with ω-3 PUFA supplementation in Dry Eye, some experts on ocular surface diseases met with the intention to establish a consensus algorithm of treatment for Meibomian Gland Dysfunction. Between some other recommended measures, they propose ω-3 PUFA supplementation when reaching a phase 2 of severity, supporting to maintain it in phases 3 and 4 of severity.

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**CONSENSUS TOWARDS THE USE OF RICH IN OMEGA-3 PUFA SUPPLEMENTS IN MEIBOMIAN GLAND DISFUNCTION**

In summary, dietetic supplementation with ω-3 PUFA induces a more stable, less evaporative, more fluid, less obstructive Meibomian sebum; this is explaining improvement of the B.U.T (tear stability) and of Schirmer test (better hydration). Increased DHA presence into cell membranes of Meibomian acini as well as in the membranes of the corneal and conjunctival epithelium is inducing the synthesis of an extra quantity of glutathione, which potentiates the antioxidant defenses in the ocular surface; this last aspect explains the inhibitory effect of DHA on the activation of the NF-KB nuclear factor, that opposes to the amplification of the inflammatory response. Predominance of ω-3 PUFA over ω-6 PUFA in cell membranes of both types of cells does favor synthesis of anti-inflammatory eicosanoids (prostaglandins, thromboxanes, leukotrienes), which mitigate inflammation and bothering Dry Eye symptoms. For this reason, oral supplementation with ω-3 PUFA in evaporative Dry Eye is offering promising results when associated to treatment with artificial tears.

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**POSITIVE OMEGA-3 PUFA INFLUENCES ON THE MEIBOMIAN LIPIDS**

- Modifies physical Meibomian lipid properties:
  - More fluidity (less possible obstructions)
  - More stable (improves B.U.T.)
  - Less evaporative (improves Schirmer T.)
  - Ocular Surface anti-inflammatory effect
  - Fights against environmental oxidative stress
  - Alleviates dry eye bothering symptoms
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28. DHA RICH PHOSPHOLIPIDS IN THE POLAR LIPID LAYER OF THE TEAR FILM

By analyzing the Meibomian lipid composition obtained by expression of the Meibomian glands, we can evaluate the presence of its different components. Polar lipids (16%) correspond with the cell membrane phospholipids in the accini. They are important in order to confer their fluidity to the mix of lipids, and to avoid obstructions in the duct or in the draining orifices. The polar lipid fraction is located on the aqueous phase of the tears, which are the sustentation base of the much thicker non-polar lipid phase, responsible of the anti-evaporative effect. Modifying our diet we can get an intense influence on them. This explains the elongation of the B.U.T. (improved the stability), and the Schirmer test improvement (better hydration).

29. OUR OWN CLINICAL RESEARCH IN DRY EYE

With the intention to confirm those findings and to evaluate if supplementation with our DHA-TG associated to antioxidant as Vitamins C and E, were offering an improvement on the parameters already mentioned: clinical, subjective and biochemical, we have designed different trials on varied groups of patients suffering different types of Dry Eye. We have administered a dose of between 2 to 3 capsules/day of BRUDYSEC 1,5 g® during 3 months to one group, and have not given any supplementation to another group. As objective parameters we have evaluated B.U.T, Schirmer Test and blinking frequency. Signs have been evaluated by using a validated OSDI questionnaire. Cytokines present (inflammatory proteins) in a sample of reflex tears have been analyzed before, and 3 months after initiating oral supplementation. Presence of A vitamin is justified due to its property facilitating mucous epithelial regeneration as Dry Eye is also affecting the ocular conjunctiva.

30. CLINICAL TRIAL IN MODERATE DRY EYE AND DHA-TG SUPPLEMENTATION

A first trial done at Dr. Peset Hospital in Valencia has evaluated utility of BRUDYSEC 1,5 g®, 2 capsules per day during 3 months with the recruitment of 66 patients: 30 patients suffering moderate Dry Eye and 36 healthy controls. 50% in each group have been supplemented. Each capsule was including 350mg of pure DHA-TG plus vitamins (A, E, C) and minerals (Se, Mn, Cu, Zn), and some other nutrients. It has been observed a significant improvement in all the clinical-objective parameters, also in the subjective clinical signs, and in the biochemical markers of inflammation present in tears.
31. **SIGNIFICANT CLINICAL IMPROVEMENT AFTER 3 MONTHS**

It was detected a significant improvement of the B.U.T. and Schirmer test in the supplemented patients suffering Dry Eye, compared with the non supplemented patients with Dry Eye. There is also an improvement in both parameters in the supplemented healthy controls when comparing them with the non supplemented. Results are indicating an improvement in tear stability, a reduction in tear evaporation and an improvement in hydration of the ocular surface.

![BRUDYSEC® 1,5 CLINICAL RESULTS AFTER 3 MONTHS OF ORAL SUPPLEMENTATION](image)

- **TEST DE SCHIRMER**: There is a significant improvement in the supplemented Dry Eye patients group (8.35±2.82 vs 4.36±0.59) Also in the supplemented control group (11.35±2.46 vs 8.35±0.59) (N=36)
- **FLUORESCINE B.U.T.**: There is a significant improvement in tear stability in the supplemented Dry Eye group (17.25±3.18 vs 4.71±1.71) also in the supplemented control group (15.26±4.75 vs 12.24±4.22) (N=36)

32. **SIGNIFICANT IMPROVEMENT OF SYMPTOMS AFTER 3 MONTHS**

There is also a significant improvement of subjective bothering symptoms measured with a validated OSDI questionnaire. After 3 months, the supplemented Dry Eye group of patients got 64% less bothering symptoms than the non-supplemented Dry Eye group. Patients got clearly alleviated.

![BRUDYSEC 1,5 CLINICAL RESULTS: OSDI SEVERITY QUESTIONNAIRE](image)

- 100% of Dry Eye patients (n=30) reported dry eye initial symptoms, which disappeared or significantly improved in 64% of the supplemented Dry Eye group after 3 months of supplementation

33. **SIGNIFICANT REDUCTION OF THE TEAR INFLAMMATORY MARKERS**

Analyzing the expression of inflammatory markers in reflex tear samples, before and after 3 months of supplementation, it can be seen a significant reduction of 3 cytokines only in the supplemented Dry Eye patients compared with the non-supplemented. These are interleukins IL-1β, IL-6, IL-10, which show also reduced in the supplemented control group, but not in the non-supplemented control group. This is to say, that oral supplementation with ω-3 PUFA (DHA-TG) has been able to mitigate the inflammatory response derived from the ocular surface dryness situation.

![EFFECTS ON INFLAMMATORY CYTOKINE TEAR EXPRESSION AT ONSET AND 3 MONTHS AFTER BRUDYSEC 1,54 SUPPLEMENTATION](image)
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Again, with the intention to understand the pathogenic mechanisms of ocular dryness suffered by Primary Chronic Glaucoma patients under topical anti-glaucoma treatment, it has been analyzed the Omega-3 PUFA supplementation effects on the expression of inflammatory and immune response molecules in pure Dry Eye patients and in glaucoma patients. 50% of the patients have been supplemented included in each group with 2 capsules per day of BRUDYSEC 1.5 g® during 3 months. There were clear differences obtained in the clinical parameters (F BUT and Schirmer) in the 3 evaluated groups comparing with results previous to initiating supplementation. Glaucoma patients show a lower level of ocular dryness than the group of patients with pure Dry Eye, and in both cases there are significant differences compared with the healthy control group.

34. **CLINICAL TRIAL IN DRY EYE DERIVED FROM GLAUCOMA TREATMENT. SUPPLEMENTATION WITH DHA TRIGLYCERIDE**

Again, with the intention to understand the pathogenic mechanisms of ocular dryness suffered by Primary Chronic Glaucoma patients under topical anti-glaucoma treatment, it has been analyzed the Omega-3 PUFA supplementation effects on the expression of inflammatory and immune response molecules in pure Dry Eye patients and in glaucoma patients. 50% of the patients have been supplemented included in each group with 2 capsules per day of BRUDYSEC 1.5 g® during 3 months. There were clear differences obtained in the clinical parameters (F BUT and Schirmer) in the 3 evaluated groups comparing with results previous to initiating supplementation. Glaucoma patients show a lower level of ocular dryness than the group of patients with pure Dry Eye, and in both cases there are significant differences compared with the healthy control group.

35. **SIGNIFICANT CLINICAL IMPROVEMENT AFTER 3 MONTHS**

Results from the supplemented group of Glaucoma patients suffering ocular dryness, after 3 month. It can be seen a significant improvement in BUT, Schirmer test, and also the bothering subjective symptoms of eye dryness. This is important, because bothering symptoms derived from the associated ocular dryness can induce a bad completion of glaucoma treatment, being the cause of a poor IOP control.

36. **SIGNIFICANT REDUCTION OF TEAR INFLAMMATORY MARKERS AFTER 3 MONTHS**

Again we can see, after 3 months of supplementation it exists a significant reduction of some interleukins as IL-6 and TNF-α. Results indicate an inflammatory process improvement associated to DHA-triglyceride supplementation. The ocular surface process suffered by patients under chronic topical glaucoma treatment does promote cytokine expression. A clear benefit of supplementation is obtained in this group of patients.
Effects of DHA plus antioxidant vitamins on oxidation and inflammatory parameters of a working population
Alfredo Filibelles, Carmen Galbis-Estrada, et al. Pending publication

- Workers at Valencia Social Security Treasury N = 80 intensive users of video-screen terminals T. Control Group none-exposed workers N = 31
- Supplementing 50% of patients in each group with 3 caps/day with BRUDYSEC 1,5g during 3 months. Also 50% non-supplemented
- Initial versus final evaluated parameters:
  1. Schirmer Test
  2. Panoramic vision average blinking frequency
  3. Average blinking in close vision (screen use)
  4. Expression of inflammatory mediators in a reflex tear collection (cytokines, chemokines)

37. CLINICAL TRIAL IN A WORKING POPULATION SUFFERING DRY EYE. SUPPLEMENTATION WITH DHA-TRIGLYCERIDE

A total of 80 workers volunteers have been recruited intensively exposed to the use of video-screen terminals during their working period, pertaining to the Treasury of the Social Security in Valencia. Clinical parameters, and inflammation plus immune molecules expression (Cytokines), were analyzed in reflex tear samples. The object was to detect any beneficial effects derived of supplementation with BRUDYSEC 1,5 g®, an oral formulation based on micronutrients, basically DHA-TG in a daily dose of 3 capsules/day.

38. SIGNIFICANT CLINICAL IMPROVEMENT AFTER 3 MONTHS

Globally, Schirmer test showed an improvement after 3 months of supplementation, evident both in the moderately and mild Dry Eye groups.

Blinking rate, both at a close and long distance vision, showed a clear improvement after oral supplementation. Both globally taken, as only one group of supplemented Dry Eye patients, but also after separating them in two groups: mild and moderate Dry Eye.

Significant differences in various inflammatory markers could be detected comparing results before and after supplementing Dry Eye patients: reduced expression in interleukins (IL-6, IL-1β), Tumor Necrosis Factor-alpha (TNF-α), Monocyte Colony Stimulating Factor (GM-CSF).

39. SUPPLEMENTATION TRIAL IN MEIBOMIAN GLAND Dysfunction
RANDOMIZED, DOUBLE BLIND, PLACEBO CONTROLLED

Results of this Double blind and placebo-controlled trial are similar to the ones obtained in the previous trials. Significant improvement in clinical parameters including BUT, Schirmer, OSDI, lid margin redness and in the lipid expression, were obtained.

A conclusion can be obtained after completing these 4 trials: DHA-TG Supplementation is offering a clear benefit to patients suffering Dry Eye.
Antioxidant¹,², Improving fluidity³, Anti-inflammatory⁴ and quality of the lipid tear film⁵. Our clinical experience

<table>
<thead>
<tr>
<th>RESEARCH / ARTICLE</th>
<th>MATERIAL &amp; METHOD</th>
<th>ACTIVE SUPPLEMENTATION</th>
<th>SIG. LEVELS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clin Interv Aging 2013; 8:139-148; MD. Pinazo-Duran et al (H. Dr Peset Valencia)</td>
<td>Moderate Dry Eye n=30 Healthy controls n=36 50% supplemented with 2 caps / day BRUDYSEC 1,5g during 90 days</td>
<td>Schirmer improvement BUT improvement OSDI: 64% symptoms reduction Cytokines expression: IL-1β, IL-6, IL-10</td>
<td>P&lt;0.05 P&lt;0.05 P&lt;0.01</td>
</tr>
<tr>
<td>Clin Interv Aging 2013; 8:711-719; Benitez del Castillo FJ et al; Hosp Jerez de la Frontera</td>
<td>Glaucoma Dry Eye n=31 Healthy controls n=36 Pure Dry Eye n=30 50% supplemented with 2 caps / day BRUDYSEC 1,5g during 90 days</td>
<td>Schirmer improvement BUT improvement VA and Rose Bengal staining OSDI: 68% symptoms reduction Cytokines expression: IL-6, FNTa</td>
<td>P&gt;0.002 P&lt;0.02 NS P&lt;0.05 P&lt;0.05 P&lt;0.001</td>
</tr>
<tr>
<td>Treasury of the SS of Valencia; Ribelles et al; Publication pending</td>
<td>Video screen users n=80 Non users n=31 50% supplemented with 3 caps / day BRUDYSEC 1,5g during 90 days</td>
<td>Schirmer improvement Short distance blinking rate Long distance blinking rate Cytokines expression: IL-6, IL-1β, FNT-a, GM-CSF</td>
<td>P&lt;0.05 P&lt;0.05 P&lt;0.05</td>
</tr>
<tr>
<td>Clin Interv Aging 2013; Accepted; Olehík A et al; Fundación Jiménez Díaz Madrid</td>
<td>Meibomian Gland Disfunction n=60 Randomized Double Blind, Placebo controlled 3 caps / day during 90 days</td>
<td>BUT improvement OSDI: symptoms reduction Lid margin inflammation Lipid expression Schirmer improvement Ocular Surface staining</td>
<td>P&lt;0.001 P&lt;0.001 P&lt;0.01 P&lt;0.01 P&lt;0.01 NS</td>
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</table>

Now with proved clinical evidences

Proposed Supplementation Guideline
Based on clinical evidences

<table>
<thead>
<tr>
<th>Level of Evolution</th>
<th>Dosing Adjustment</th>
<th>Box Duration</th>
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<tbody>
<tr>
<td>First visit: Overt Dry Eye</td>
<td>Impregnation dose: 3 capsules/day</td>
<td>30 days</td>
</tr>
<tr>
<td>Second visit: Patient feels better</td>
<td>Adjusted dose: 2 capsules/day</td>
<td>45 days</td>
</tr>
<tr>
<td>Third visit: Patient feels better</td>
<td>Maintaining dose: 1 to 2 capsules/day</td>
<td>90 to 45 days</td>
</tr>
</tbody>
</table>
BRUDYLAB is proposing you two possible ways of facing Dry Eye in your patients

**Oral route: BRUDYSEC 1,5g**

- 2 to 3 caps/day during 3 months does offer a significant clinical improvement:
  - On tear stability (B.U.T.)
  - Tear production (Schirmer test)
  - Bothering symptoms (OSDI)
  - Reducing expression of inflammatory cytokines in tears


DHA Triglyceride offers better bioavailability and an optimal gastrointestinal tolerance

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**Topical route: BRUDYAL**

- 0,1% Sodium Hyaluronate solution PRESERVATIVES FREE
- Highly Hydrating and lubricating ocular surface at an affordable price for all.

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**Bibliography**

(1) José A. Villegas et al; Dept. Fisiología; Universidad Católica de San Antonio, Murcia; Protección del daño oxidativo en el DNA tras el ejercicio intenso; Comunicación presentada en el 30 Congreso Mundial de Medicina del Deporte, Barcelona, 18-23 Noviembre 2008.

(2) J. Martínez-Soro, A. Domingo; Effect of dietary DHA supplementation on sperm DNA integrity; Fertility & Sterility 2010; 94:5253-5258; Comunicación presentada en el 66th Annual Meeting of the ASRM (2010).

(3) Rencz MC et al; Essential fatty acids for dry eye: A review; Contact Lens Anterior Eye 2010; 33(2):69-94.

